PCT/NL 2004 / 000760

Europäisches Patentamt | European Patent Office | Office européen des brevets

REC'D 19 NOV 2004
WIPO PCT

Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

03078396.3

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

Anmeldung Nr:
Application no.: 03078396.3
Demande no:

Anmeldetag:
Date of filing: 29.10.03
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

Academisch Ziekenhuis bij de Universiteit
van Amsterdam
Academisch Medisch Centrum,
Meibergdreef 15
1105 AZ Amsterdam ZO
PAYS-BAS

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Use of a deoxynojirimycin derivate or a pharmaceutically salt thereof

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

A61K31/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL
PT RO SE SI SK TR LI

Title: Use of a deoxynojirimycin derivative or a pharmaceutically salt thereof

FIELD OF THE INVENTION

The present invention relates to the use of a deoxynojirimycin derivative, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of various diseases in which the synthesis of glucosylceramide and/or other glycosphingolipids play a role. Such diseases include insulin resistance (i.e. diabetes mellitus type II), hyperpigmentation, fungal diseases and microbacterial infections. In particular the invention relates to the use of a deoxynojirimycin derivative, or a pharmaceutically acceptable salt thereof, for the preparation of medicaments for the treatment of insulin resistance, i.e. diabetes mellitus type II.

The incidence of diabetes mellitus type II is dramatically increasing in the Western World. The primary underlying defect is an impaired uptake of glucose from the bloodstream by muscle and adipose tissue as the result of a reduced sensitivity to mobilize GLUT4 transporters to their cell surface in response to insulin. It is already known for many years that increased concentrations of fatty acids in muscle are associated with aberrant glucose homeostasis. Conversely, the improvements of glucose homeostasis induced by PPAR gamma agonists and rexinoids are associated with altered partitioning of fatty acids, i.e. redistribution of fatty acids to adipose tissue and relative depletion of muscle fatty acid uptake and metabolism. Poorly understood, however, is the molecular mechanism by which lipotoxicity in the muscle causes onset and progression of diabetes. Further insight in this matter will therefore assist in improving/developing medicaments for treating insulin resistance.

MOLECULAR MECHANISM OF LIPOPATHOGENESIS

Research activities on glycosphingolipids and diabetes mellitus type II in the Department of Biochemistry at the Academic Medical Center/University

of Amsterdam has recently led to an unexpected new insight in the lipopathogenesis of diabetes mellitus type II. The underlying mechanism is described in detail below.

ROLE FOR GLYCOSPHINGOLIPIDS IN ACQUIRED INSULIN RESISTANCE

A role is hypothesized for (glyco)sphingolipids in the pathogenesis of diabetes. This thought stems from the ignored fact that palmitate is the essential building block of the ceramide moiety in sphingolipids: the first step of their biosynthesis involves the transfer of palmitate to serine, catalyzed by serine palmitoyltransferase, see Figure 1. The rate of synthesis of sphingolipids in the liver is highly dependent on the concentration of palmitate. Importantly, this could be experimentally confirmed for cultured muscle cells (smooth muscle cells, myoblasts): addition of 0.1, 0.5, 1.0 mM palmitate in the culture medium led to proportional increases in the synthesis of glycosphingolipids, as revealed by increased incorporation of radio-labelled serine in these structures.

This finding prompted a more detailed examination of the possibility that actually (glyco)sphingolipids mediate the lipotoxicity in muscles that underlies diabetes. It has recently been evidenced that GM3 (the most simple ganglioside at the cell surface, see Figure 2) may impair insulin signalling. In this respect it is observed that the concentration of GM3 at the cell surface appears to regulate the uptake of glucose in response to insulin by negatively interfering with multi-clustering of insulin receptors. Moreover, high concentrations of GM3 are associated with reduced mobilization of GLUT4 to the cell surface. Conversely, reduction of GM3 is associated with enhanced insulin sensitivity (see Yamishita et al. Proc Natl Acad Sci USA (2003) 100, 3445-9 Enhanced insulin sensitivity in mice lacking ganglioside GM3; Tagami et al. (2002) J Biol Chem 277,3085-92 Ganglioside GM3 participates in the pathological conditions of insulin resistance). We postulate that at obese

conditions, palmitate levels are chronically high and that therefore the formation of glycosphingolipids in adipocytes as well as muscle cells will occur at increased rates, favouring insulin resistance. The connection between increased concentration of palmitate in muscle as driving force for the increased local glycosphingolipid synthesis (including GM3) and insulin resistance (see Figure 3) has not yet been recognised by others.

CRUCIAL ROLE OF GLUCOSYLCERAMIDE SYNTHASE

It was further realised that the concentration of GM3 and other gangliosides at the cell surface is dependent on the activity of glucosylceramide synthase (the synthesis of glucosylceramide), the rate limiting step in ganglioside synthesis (see Figure 3). This enzyme catalyzes the formation of glucosylceramide from ceramide and UDP-glucose. The Km values of both its substrates (ceramide and UDP-glucose) are in the physiological range. We show that glucosylceramide synthase is a key regulatory enzyme with respect to insulin sensitivity. Increases in its activity have been observed and reported previously in response to inflammatory cytokines (TNF-alfa), steroid hormones, saturated fatty acid, and viral infection. It has now surprisingly been found that the changes in glycosphingolipid synthesis have an impact on the promotion of diabetes mellitus type II (see Figure 4). This finding that lipopathogenesis impacts diabetes type II shows that inhibition of glucosylceramide synthase activity exerts a beneficial, anti-hyperglycaemic effect.

NOVEL USE OF IMUNOSUGAR-BASED INHIBITORS

It has become clear that deoxynojirimycins, a particular category of iminosugars, are suitable agents to reduce glycosphingolipid synthesis by the inhibition of the synthesis of glucosylceramide. Further, considerable hands-on expertise has been obtained with the safety of iminosugar administration in man.

N-butyl-deoxynojirimycin has been recently registered as drug for the treatment of type 1 Gaucher disease. A clinical study, largely undertaken at the Academic Medical Center in collaboration with the University of Cambridge, revealed that the drug is tolerated in the majority of patients, at least up to 5 years. Despite the fact that glycosphingolipid synthesis is only very moderately (20-30%) inhibited by 100 mg TID N-butyl-deoxynojirimycin, some Gaucher patients develop, however, serious intestinal complaints and occasionally alarming peripheral neuropathy. At higher doses of N-butyl-deoxynojirimycin these adverse events occur even more frequently. We postulate that the poor specificity of N-butyl-deoxynojirimycin with respect to inhibition of glucosidases and glucosyltransferases contributes to these undesired side-effects. As shown in Table 1, N-butyl-deoxynojirimycin is also a very potent inhibitor of intestinal glycosidases. We postulate that this inhibiting effect results in at least part of the intestinal complaints of patients. It is an inhibitor of lysosomale alfa-glucosidase and glucocerebrosidase. We postulate that this effect results in the associated risk for pathological intralysosomal accumulation of glycogen and glucocerebroside in lysosomes. At concentrations required to significantly lower GM3 in (pre)diabetic persons, adverse events will undoubtedly occur when use is made of N-butyl-deoxynojirimycin.

SUMMARY OF THE INVENTION

Accordingly, the present invention relates to the use of a deoxynojirimycin derivative, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of insulin resistance, which deoxynojirimycin derivative or salt displays a capacity for the inhibition of the synthesis of glucosylceramide and/or other glycosphingolipids and a capacity for the inhibition of the activity of glycosidases.

The deoxynojirimycin derivates that can suitably be used in accordance with the present invention have been described in WO 98102161

and J. Biol. Chem. 273, 26522-27(1998), which documents are both hereby incorporated by reference.

Preferably, the deoxynojirimycin derivative comprises an apolar side chain linked to the nitrogen atom of deoxynojirimycin.

Preferably, the apolar side chain comprises a large hydrophobic moiety which is derived from a polycyclic alcohol containing three or more rings each sharing two or more carbon atoms with another ring and which is capable of inserting in lipid bilayers.

Preferably, the large hydrophobic moiety is linked to said nitrogen atom of the deoxynojirimycin by means of a spacer comprising an alkoxy polyalkylene or polyalkylene chain of from 3 to 8 carbon atoms. More preferably, the large hydrophobic moiety is derived from a compound selected from the group consisting of adamantanemethanol, cholesterol, ß-cholestanol, adamantanol and 9-hydroxyphenanthrene.

The word 'spacer' refers to any bivalent moiety or group capable of linking a hydrophobic group to the N atom of deoxynojirimycine.

DESIGN OF A SPECIFIC INHIBITOR

In particular N-(5-adamantane-1-yl-methhoxy-pentyl)deoxynojirimycin; formerly also known as AMP-DNM, has been identified as a very potent inhibitor of the glucosylceramide synthase (IC50 ~ 100 nM in cultured cells), which is explained by the fact that molecular modelling of the enzyme structure reveals that it has a near perfect fit in the catalytic site. N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin has various additional attractive features. In addition the compound shows a good oral bioavailability.

Hence, the deoxynojirimycin derivative is preferably N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin or a derivative or analogue thereof.

OTHER THERAPEUTIC APPROACHES

It has further been found that the above-mentioned deoxynojirimycin derivatives can be used for treating hyperpigmentation and/or inflammatory processes in the skin. Therefore, the present invention also relates to the use of a deoxynojirimycin derivative, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of hyperpigmentation and/or inflammatory processes in the skin. Preferably, the present invention relates to the use of a deoxynojirimycin derivative, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of hyperpigmentation and/or inflammatory processes in the skin, which deoxynojirimycin derivative or salt displays a capacity for the inhibition of the synthesis of glucosylceramide and/or other glycoshingolipids.

It has also been found that the above-mentioned deoxynojirimycin derivatives can suitably be used in the treatment of fungal diseases. Hence, the present invention further relates to the use of a deoxynojirimycin derivative, or pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of a fungal disease. Preferably, the present invention further relates to the use of a deoxynojirimycin derivative, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of a fungal disease, which deoxynojirimycin derivative or salt displays a capacity for the inhibition of the synthesis of glucosylceramide and/or other glycoshingalipids.

Further, it has been found that the above-mentioned deoxynojirimycin derivatives can suitably be used in the treatment of microbacterial infections. The present invention relates therefore also to the use of a deoxynojirimycin derivative, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of a microbacterial infection. Preferably, the present invention relates therefore also to the use of a deoxynojirimycin derivative, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment

of a microbacterial infection, which deoxynojirimycin derivative or salt displays a capacity for the inhibition of the synthesis of glucosylceramide and/or other glycoshingolipids. It has also been found that the above-mentioned deoxynojirimycin derivatives can suitably be used in the treatment of overweight and obesity. The present invention relates therefore also to the use of a deoxynojirimycin derivative, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of overweight and obesity. Preferably, the present invention relates therefore also to the use of a deoxynojirimycin derivative, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of overweight and obesity, which deoxynojirimycin derivative or salt displays a capacity for the inhibition of the synthesis of glucosylceramide and/or other glycoshingolipids.

The present invention also relates to a method of treatment of an individual suffering from a disease selected from the group consisting of insulin resistance, hyperpigmentation and/or inflammatory processes in the skin, fungal diseases, overweight and obesity, and microbacterial infections, comprising administrating to said individual an effective amount of a medicament comprising a deoxynojirimycin derivative, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

The selection of a suitable route of administration and suitable formulations of pharmaceutical compositions is within the normal skills of the persons skilled in the art. Examples of suitable administration routes are parent (intravenous, subcutaneous, intramuscular) injections or infusions, oral ingestion, and topical application.

EXPERIMENTAL PART

Animal studies have indicated that N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin is well tolerated and does not result in any overt pathology. In view of this, it was examined whether N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin is able to reduce GM3 levels at the surface of

cells. For this purpose, cells were exposed for 3 days to N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin, and GM3 expression was assessed by FACS analysis with a monoclonal antibody specific to the ganglioside. In various cell types (including muscle cells), surface GM3 was potently reduced. The IC50 value for surface GM3 reduction was about 250 nM. Next the ability of N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin to inhibit various glycosidases and glucosyltransferases was studied and compared to that of other known and designed iminosugars. As will be clear from Table 1, N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin is from all iminosugars investigated by far the most potent and specific inhibitor of the synthesis of glucosylceramide. Variations in hydrophobic moiety (adamantanemethyl, adamantanyl, phenantryl, cholesteryl and cholestanyl) and spacer (length 3-8 carbon atoms and presence of carbonyl moiety) indicated that N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin is the most optimal inhibitor.

Next, the ability of N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin to inhibit the synthesis of various glycosidases and glucosyltransferases was studied and was compared to that of other known and designed iminosugars.

The value of N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin as suppressor of hyperglycaemia was studied in mouse models of obesitas. C57BL/6J mice (ob-/-, db -/-, and +/+) were orally treated with 0, 25 or 100mg N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin /kg body weight daily. Plasma glucose, water intake and food intake were monitored. The administration of N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin by food resulted in the obese and diabetes mice (in dose-dependent) in reductions of blood glucose and water intake. See Figures 6-7. Even in normal mice a small reduction in blood glucose was already noted.

Furthermore, a major reduction in urinary glucose was noted after 2 weeks treatment, particularly in ob-/ob- mice at the highest dose of N-(5-adamantane 1-yl-methoxy-pentyl)deoxynojirimycin. See Figure 8.

Similar effects as presented in Figures 5-8 were noted in various independent experiments. These results are a firm proof that N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin can be used as an anti-hyperglycaemic agen for the treatment of diabetes mellitus type II.

In FVB and C57BL/6J mice the effect of oral administration of N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin on (glyco)sphingolipids wa studied to validate the mode of action of N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin. Using a very sensitive and accurate HPLC-based method, ceramide and glucosylceramide levels in treated (100 mg N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin /kg) and matched untreated mice were compared. Liver, muscle and brain were analyzed. No changes in ceramide concentration in any of these tissues were noted. Concomitantly the liver glucosylceramide was reduced by 80% and muscle glucosylceramide by 50%. No changes were detected in brain galactosylceramide concentration. TLC analysis was employed to monitor the effects on gangliosides. A marked reduction was noted in the liver GM3 (see Figure 9). A reduction was also observed in muscle GM3 (not shown). No changes were noted in brain gangliosides or sulfatide (Figure 9).

The findings on glycoshingolipids levels in the liver are consistent with the finding that plasma levels of N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin are ~1 uM (4 weeks 100mg N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin /kg orally, as determined by enzymatic method). Apparently, N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin poorly penetrates in the brain but is capable to reach muscle tissue.

In conclusion, the changes in glycosphingolipids exerted by the oral administration of N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin

correlate nicely with the anti-hyperglycaemic effect of the compound. These findings clearly show the attractiveness of the present invention.
Other aspects and advantages of the present invention will be understood upon consideration of the following illustrative experiments.

Example 1: In cultured cells the insulin signaling improvement is revealed by the phosphorylation of S6 kinase in hepatocytes and glucose uptake by muscle cells under influence of N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin. Initial experiments already confirmed this effect.

Example 2: The increase in insulin sensitivity under the influence of N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin in high fat diet fed rats can be determined using euglycemic hyperinsulinemic clamps.

The realization of the crucial role of palmitate and glycosphingolipids in insulin resistance in muscle of diabetes type II patients rendered a rationale for the development of an anti-hyperglycaemic drug. N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin shows very attractive pharmacological features (bioavailability, lack of metabolism) and its oral administration does not result in any overt pathology. Importantly, N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin is able to interfere directly in the pathological cascade by lowering GM3 levels in muscle of mice. Only metabolism of glycosphingolipids is affected, not that of galactosphingolipids and sphingomyeline. The inhibition of glucosylceramide formation does also not effect ceramide concentrations. Oral administration of N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin results in the desired reduction of blood and urinary glucose in obese mice that suffer from diabetes mellitus type II. Proof of concept for the use of N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin as anti-hyperglycaemic agent was thus obtained.

FURTHER SELECTION OF OPTIMAL ANTI-HYPERGLYCAEMIC IMINOSUGAR

Another approach to intervene in diabetes mellitus type II is based on buffering the uptake of food-derived saccharide in the gastrointestinal tract by inhibition of intestinal glycosidases. Synthetic inhibitors of sucrase (Acarbose, N-hydroxyethyl-deoxynojirimycin) are based on this concept and are registered antidiabetic drugs. N-hydroxyethyl-deoxynojirimycin is the most potent antidiabetic agent which exerts its activity through inhibition of sucrase.

The big disadvantage of potent synthetic inhibitors of intestinal glycosidases like N-hydroxyethyl-deoxynojirimycin and Acarbose is, however, that they can cause severe intestinal complaints. A potent inhibition of intestinal glycosidase results in accumulation of osmotic active sugars in the gastrointestinal lumen and favours enterobacterial growth, both contributing to spasms and diarrhoea. The potent intestinal glycosidase inhibitors N-hydroxyethyl-deoxynojirimycin and Acarbose are therefore not very well tolerated by many individuals, resulting in a poor compliance and limited application.

Since a moderate inhibition of intestinal glycosidases may exert an additional beneficial effect in individuals with diabetes mellitus type II without causing the undesired intestinal complaints caused by potent inhibitors, the iminosugars were examined on this aspect. It was found that N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin is also able to inhibit intestinal sucrase (IC50 ~4.5 mM). The inhibition is, however, far less harsh as exerted by N-butyl-deoxynojirimycin and N-hydroxyethyl-deoxynojirimycin. Similar findings were made with respect to maltase-glycoamylase inhibition. Again N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin behaved favourably when compared to N-hydroxyethyl-deoxynojirimycin and N-butyl-deoxynojirimycin. The structures of these compounds are shown in Figure 10.

CRITERIA USED TO IDENTIFY A SUITABLE IMINOSUGAR AGENT FOR THE TREATMENT OF DIABETES MELLITUS TYPE II.

To identify the most desirable iminosugar-based agent for treatment of diabetes mellitus type II, the following set of criteria was used: the compound should be highly bioavailable when orally administered (favouring a hydrophobic character as shown by N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin); the compound should be a potent inhibitor of glucosylceramide synthase; the compound should be specific and inhibit poorly the lysosomal glycosidase, alfa-glucosidase and glucocerebrosidase; the compound should not be an aggressive inhibitor of intestinal sucrase; and the compound should be metabolically inert. Fulfilment of these criteria results in a safe, effective and well tolerated anti-hyperglycaemic agent.

The deoxynojirimycin derivates used in accordance with the present invention meet these criteria. Especially, N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin and structurally closely related compounds were found to be optimal agents. They are more attractive than N-butyl-deoxynojirimycin and N-hydroxyethyl-deoxynojirimycin in virtually every aspect.

PRODUCTION OF N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin and other IMINOSUGARS

All N-substituted iminosugars were synthesized according to the general procedure described in J. Biol.Chem, 273, 26522-27 (1998).

ENZYME ACTIVITY ASSAYS

Sucrase, lactase and maltase activities were determined using freshly isolated rat intestinal membranes. Membrane homogenates were prepared by extensive washing and homogenization using a Potter and ultrasound sonication. Membrane homogenates were incubated at 0.5 M potassium

phosphate (pH 6.5) for 120 min at 37 °C with either 25 mM sucrose, 25 mM lactose or 25 mM maltose. The reactions were stopped by exposure the sample for 2 minutes to 100 °C. Following centrifugation the glucose content of the supernatant was determined spectrophotometrically at 550 nM using the GOD-PAP procedure (Merck) according to the manufacturer's instructions. Glucocerebrosidase and alfa-glucosidase activities were determined using artificial, fluorogenic 4-methylumbelliferyl-substrates exactly as described in J.Biol.Chem, 273,26522-27 (1998).

Glucosylceramide synthase activity was determined using membrane fractions from type 1 Gaucher disease spleen as source of enzyme. For this purpose, spleen was homogenized in water, the homogenate was centrifuged at 25.000 g for 1 hour, resuspended in water and centrifuged again. Following resuspension, ultrasound sonication was used to prepare a homogenate. The membrane homogenate was incubated with conduritol B-epoxide (1 mM) to covalently inhibit any residual glucocerebrosidase activity. The membrane homogenate was subsequently incubated with 0.02 mM C6-NBD-ceramide complexed to albumin for 30 minutes in 0.1M potassium phosphate (pH 7.5) buffer containing 10 mM magnesium chloride and 20 mM UDP-glucose. The reaction was stopped by addition of a mixture of chloroform and methanol. Following a Folch extraction, the lower chloroform phase was evaporated under nitrogen and remaining lipids were dissolved in chloroform:methanol (2:1 v/v). The dissolved lipids were subjected to thin layer chromatography on HPTLC Silica plates in chloroform: methanol: water (65:25:4 v/v/v). Fluorescent NBD-containing lipids were then visualized and quantified using a STORM-imager. Formation of C6-NBD-glucosylceramide from C6-NBD ceramide was subsequently determined and reflected glucosylceramide synthase activity.

DETERMINATION OF IC50 VALUES

IC50 values were determined by exposing enzyme preparations to various dilutions of iminosugars and determining the concentration at which enzyme activity was reduced by 50%.

LIPID ANALYSES

Lipids were extracted from tissue and plasma following the well-known Bligh & Dyer procedure.

The lower (chloroform) phase was dried under nitrogen vapour. The total lipid residue was deacylated in 0.5 ml 0.1 M NaOH in methanol in a SAM-155 microwave oven for 20 minutes at 75% of maximal energy. Subsequently lipids were extracted according to the Folch procedure, and the chloroform phase was dried and lipids were resuspended in 250 ul methanol. Deacylated glycolipids were derivatised with O-phtalate and subjected to high performance liquid chromatography. HPLC analysis was conducted using a Hypersil BDS C18 column with a solvent system of methanol: 0.1 M boric acid pH 3.8 (87.5:12.5, v/v),

The upper phase of the Bligh & Dyer extraction was used for the detection of gangliosides. For this purpose, the upper phase was dried and the residue was taken up in 1 ml 0.1M NaCl, pH 4.0. The solution was passed through a C18 Sep-Pak cartridge, washed with water and the gangliosides were quantitatively eluted with chloroform:methanol (1:1). Individual gangliosides were separated on HPTLC Silica plates using chloroform:methanol:water (55:45:10 v/v/v) containing 0.2% calcium chloride. Gangliosides were subsequently visualised by spraying with the orcinol stain and quantified by densitometry.

CELL CULTURE

Cells (transformed smooth muscle cells, myoblasts, fibroblasts and freshly isolated rat hepatocytes) were cultured in RPMI medium in the presence of 10% foetal calf serum.

ANIMALS

Mice (obese-lep ^{ob} (C57Bl/6)laHsd-Lep ^{ob}); diabetes –lepr ^{db} (C57Bl/6KsOlaHsd – Lepr ^{db}); wildtype (C57Bl/6J OlaHsd) and wild type FVB (FVB/NHanHsd) were obtained from Harlan Nederland. Animals were fed with AM-II diet with or without N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin (Hope Farms Netherlands). Mice were kept in groups of five in each cage. Water consumption and food intake were determined three times weekly per cage. Body weight of individual animals was determined three times weekly. Glucose in plasma was determined with the GOD-PAP method (Merck), and glucose in urine was determined using GLUKETUR test strips (Roche).

TABLE 1

IC50 value (uM) for inhibition of:

Iminosugar	GCsynthase		GlcCer-ase	Alfa-Glu-ase	Sucrase	Maltase	Lactase
	Vitro	Vivo#					
N-butyl-deoxynojirimycin	1200	35	500	0.8	0.5	20	9
AMP-DNM	15	0.3	0.4	0.1	4.5	>25	18
N-hydroxyethyl-deoxynojirimycin	2000	nd	nd	nd	0.1	nd	nd

#:GC synthase was also determined in intact cells that were fed with C6-ND-Cer and for which formation of C6-NBD-GlcCer was monitored.
AMP-DNM: N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin
nd: not determined

IC50 values (i.e. inhibitor concentrations resulting in 50% inhibition) were determined by variation of inhibitor concentrations. Note: N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin penetrates poorly lysosomes in intact cells and IC50 values for inhibition of lysosomale glycosidase (glucocerebrosidase, acid alfa-glucosidase) are at least 20-fold higher than in vitro values.

Legends to the figures

Figure 1.
Overview of GM3 formation: rate limiting are palmitate and conversion of ceramide + UDP-glucose to glucosylceramide

Figure 2
Biosynthesis of major glycosphingolipids

Figure 3.
Concept lipo-pathogenesis muscle in diabetes mellitus type II: key regulatory role for glucosylceramide synthase.

Figure 4. Risk factors for diabetes: promoters of GlcCer formation

Figure 5. Effect of daily oral administration of x mg N-(5-ADAMANTANE-1-YL-METHOXY-PENTYL)DEOXYNOJIRIMYCIN /kg) on C57Bl6 mice (means of 5 animals)

(MZ-21= N-(5-ADAMANTANE-1-YL-METHOXY-PENTYL)DEOXYNOJIRIMYCIN)

Figure 6. Effect of daily oral administration of x mg N-(5-ADAMANTANE-1-YL-METHOXY-PENTYL)DEOXYNOJIRIMYCIN /kg) on C57Bl6 mice 0b-/0b- (means of 5 animals)

Figure 7. Effect of daily oral administration of x mg N-(5-ADAMANTANE-1-YL-METHOXY-PENTYL)DEOXYNOJIRIMYCIN /kg) on C57Bl6 mice db-/db- (means of 5 animals)

Figure 8. Effect of daily administration of MZ21 on urinary glucose after 2 wks treatment.

Figure 9. Changes in gangliosides in tissues of N-(5-ADAMANTANE-1-YL-METHOXY-PENTYL)DEOXYNOJIRIMYCIN treated mice (2 wks 100 mg/kg).

Figure 10. Structures of cited N-substituted deoxynojirimycines

Claims

1. Use of a deoxynojirimycin derivative, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of insulin resistance, which deoxynojirimycin derivative or salt displays a capacity for the inhibition of the synthesis of glucosylceramide and/or other glycosphingolipids and a capacity for the inhibition of the activity of intestinal glycosidases.

2. Use of a deoxynojirimycin derivative, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of hyperpigmentation and/or inflammatory processes in the skin.

3. Use of a deoxynojirimycin derivative, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of a fungal disease.

4. Use of a deoxynojirimycin derivative thereof, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of a microbacterial infection.

5. Use of a deoxynojirimycin derivative thereof, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of overweight and obesity.

6. Use according to any one of claims 1-5, wherein said deoxynojirimycin derivative comprises an apolar side chain linked to the nitrogen atom of the deoxynojirimycin.

7. Use according to claim 6, wherein said apolar side chain comprises a large hydrophobic moiety.

8. Use according to claim 6 or 7, wherein said large hydrophobic moiety is linked to said nitrogen atom of the deoxynojirimycin by means of a spacer comprising an alkoxy polyalkylene or polyalkylene chain of from 3 to 8 carbon atoms.

9. Use according to any one of claims 5-8, wherein said large hydrophobic moiety is derived from a compound selected from the group consisting of adamantanemethanol, cholesterol, β-cholestanol, adamantanol and 9-hydroxyphenanthrene.

10. Use according to any one of claims 5-9, wherein said apolar side chain is derived from a polycyclic alcohol containing three or more rings each sharing two or more carbon atoms with another ring and is capable of inserting in lipid bilayers, and wherein the spacer comprises an alkoxy polyalkene or polyalkene chain of from 3 to 8 carbon atoms.

11. Use according to any of claims 1-10, wherein said deoxynojirimycin comprises N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin or a derivative or an analogue thereof.

12. Method of treatment of an individual suffering from insulin resistance, comprising administrating to said individual an effective amount of a medicament comprising a deoxynojirimycin derivative, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, which deoxynojirimycin derivative or salt displays a capacity for the inhibition of the synthesis of glucosylceramide and/or other glycosphingolipids and a capacity for the inhibition of the activity of intestinal glycosidases.

13. Method of treatment of an individual suffering from a disease selected from the group consisting of hyperpigmentation and/or inflammatory processes in the skin, fungal diseases, overweight and obesity, and microbacterial infections, comprising administrating to said individual an effective amount of a medicament comprising a deoxynojirimycin derivative, o a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

Abstract

The invention relates to the use of a deoxynojirimycin derivative, or pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of insulin resistance, hyperpigmentation and/or inflammatory processes in the skin, a fungal disease, overweight and obesity, or a microbacterial infection.

Figure 1.

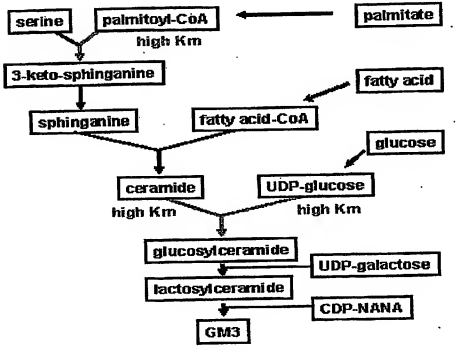


Figure 2.

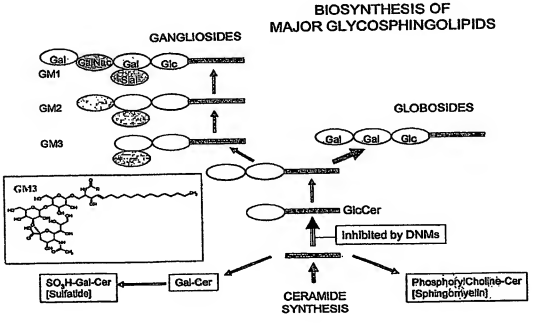


Figure 3.

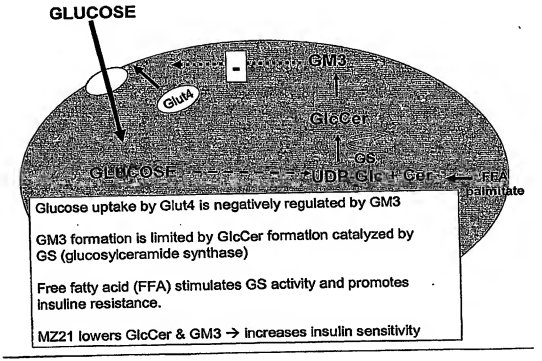


Figure 4.

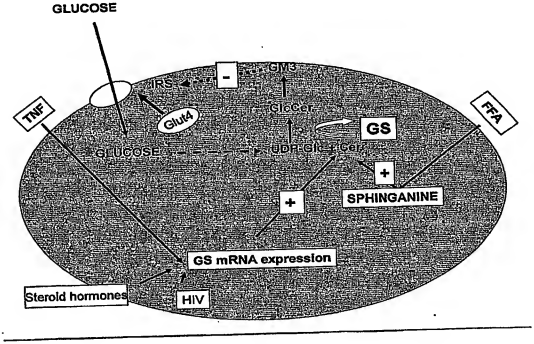


Figure 5.

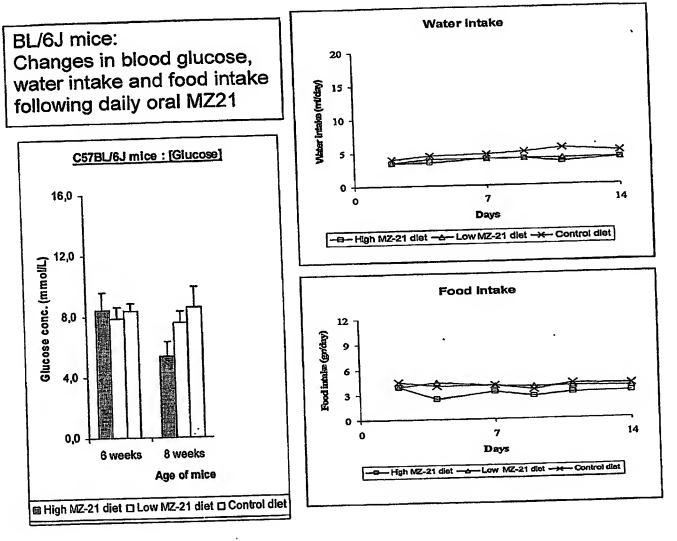

BL/6J mice:
Changes in blood glucose, water intake and food intake following daily oral MZ21
C57BL/6J mice : [Glucose]
Glucose conc. (mmol/L)
16,0
12,0
8,0
4,0
0,0
8 weeks
8 weeks
Age of mice
High MZ-21 diet
Low MZ-21 diet
Control diet
Water Intake
Water intake (ml/day)
20
15
10
5
0
0
7
14
Days
High MZ-21 diet
Low MZ-21 diet
Control diet
Food Intake
Food intake (gm/day)
12
9
6
3
0
0
7
14
Days
High MZ-21 diet
Low MZ-21 diet
Control diet

Figure 6.

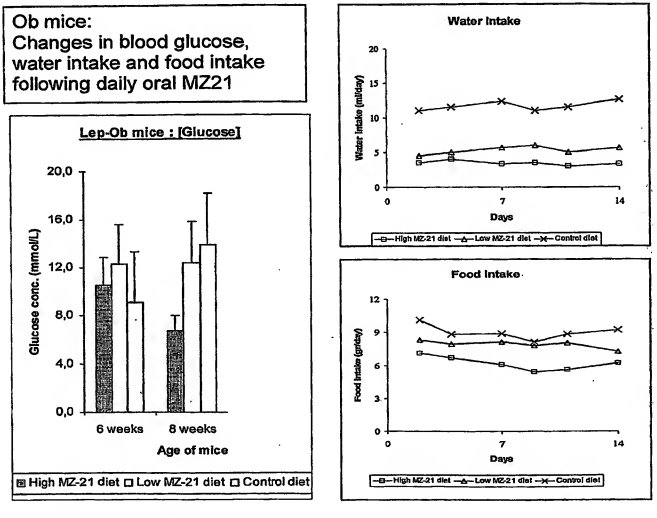
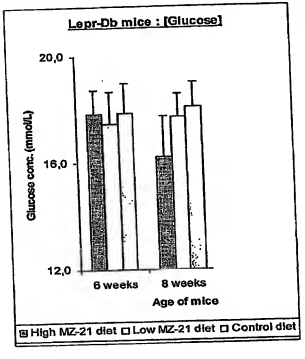
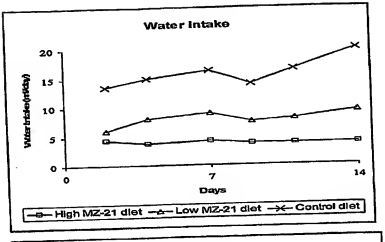

Ob mice:
Changes in blood glucose, water intake and food intake following daily oral MZ21
Lep-Ob mice : [Glucose]
Glucose conc. (mmol/L)
20,0
16,0
12,0
8,0
4,0
0,0
6 weeks
8 weeks
Age of mice
High MZ-21 diet
Low MZ-21 diet
Control diet
Water Intake
Water Intake (ml/day)
20
15
10
5
0
0
7
14
Days
High MZ-21 diet
Low MZ-21 diet
Control diet
Food Intake
Food Intake (gr/day)
12
9
6
3
0
0
7
14
Days
High MZ-21 diet
Low MZ-21 diet
Control diet

Figure 7.

Db mice:
Changes in blood glucose, water intake and food intake following daily oral MZ21





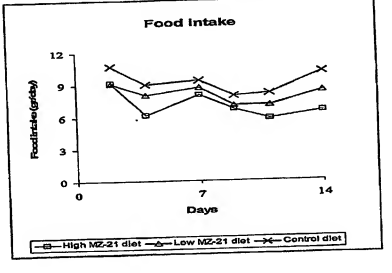


Figure 8.

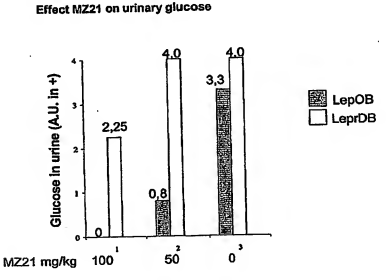
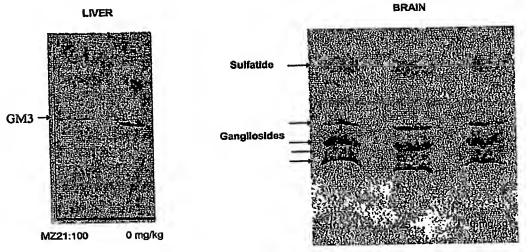

Effect MZ21 on urinary glucose
Glucose in urine (A.U. in +)
0
1
2
3
4
0
2,25
0,8
4,0
3,3
4,0
MZ21 mg/kg
100
50
0
1
2
3
LepOB
LeprDB

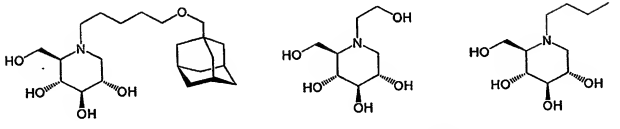
Figure 9.



MZ-21 reduces liver GM3

MZ-21 has no effect on brain gangliosides, arylsulfatide or galactosylceramide

Fig 10.



N-(5-adamantane-1-yl-methoxy-pentyl)deoxynojirimycin N-hydroxyethyl-deoxynojirimycin N-butyl-deoxynojirimycin